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GENETIC AND ENVIRONMENTAL BASES OF TOBACCO-RELATED BEHAVIOR

Introduction

The flood of reports relating cigarette smoking to lung cancer and other diseases in the early 1950's resulted in the widespread acceptance of the concept that cigarette smoking causes the sickness. It has been realized that this is an incomplete explanation, for not everyone who smokes, even heavily, contracts lung cancer, respiratory disease or heart disease. Other factors must be operative, and considerable effort has been exerted to detect and define these "other factors," mainly through an epidemiological approach.

The rather loosely used concept of genetic predisposition has been offered as an hypothesis to explain individual differences in the relationship between smoking and disease, especially since monozygous twin studies have become more widely known. According to this hypothesis, smoking is the precipitating factor, but some genotypes are considered to confer protection against this precipitant. A converse hypothesis was promulgated by R. A. Fisher (1958a). He suggested that the genetic make-up which results in cigarette smoking being pleasurable might be the same genotype which causes predisposition to lung cancer.

In fact, evidence does exist that is strongly suggestive of an hereditary influence on smoking behavior. Fisher (1958b) reported that of 51 pairs of adult male monozygous twins, 33 pairs were concordant (9 pairs non-smokers, 22 pairs cigarette smokers and 2 pairs cigar smokers), while of 31 pairs of adult male dizygous

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twins, only 11 pairs were concordant. Fisher concluded that "there can therefore be little doubt that the genotype exercises a considerable influence on smoking, and on the particular habit of smoking adopted."

The conclusion was challenged on the basis that the cultural development of monozygous twins is such that they would be expected to behave alike, and that it would be no surprise if they did. This superficially compelling argument founders when monozygous twins reared apart are considered. Shields (1962) investigated the smoking habits of dizygous twins and of monozygous twins reared apart and together, with the following results:

	Smoking Habit	
	Concordant	Discordant
Dizygous twins	9	9
Monozygous twins		
Reared apart	23	4
Reared together	21	5
	44	9

The concordance results are similar for monozygous twins whether reared apart or together, and they are consistent with the data reported by Fisher. This conclusion is supported by other twin studies (Friberg et al., 1959; Todd & Mason, 1959; Raaschou-Nielsen, 1960). There are also other suggestive lines of evidence such as parent-offspring similarity (Salber & Abelin, 1967), differences between smokers and non-smokers in tasting ability for PTC (Thomas & Cohen, 1960) and their relative proportion of α, β and θ wave activity in EEG's (Brown, 1973).

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The proposed research program will be concerned with further investigation of the genetic basis of tobacco-related behavior. Specific research questions will include:

(1) Is it possible to demonstrate unequivocal genetic involvement in smoking behavior?

(2) What is the relative magnitude and nature of the genetic control?

(3) Is it possible to determine the genetically influenced neurophysiological and pharmacological correlates of tobacco-related behavior?

(4) Is it possible to discriminate between smokers and non-smokers without reference to smoking behavior?

Data obtained from investigations into these questions should contribute substantially to understanding one aspect of the "Fisher hypothesis"--the hereditary influence on smoking behavior. In addition, we shall eventually be able to address the total hypothesis directly. This could be accomplished by locating a population within which social or religious beliefs prevent or impede smoking. Individuals from this group, discriminated as having the attributes of smokers in the general population, can be compared for disease incidence with those having attributes of non-smokers.

In discussing the proposed methodology, we must first state that the three areas of activity identified below are not mutually exclusive. The same questionnaires will be used in all studies involving humans; the several populations studied, each having its own main emphasis, will provide information for the other areas of activity; the biochemical and pharmacological procedures used in the

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animal studies will be paralleled when feasible in the human studies; and so on. The progress of the research will be the subject of scheduled discussion among the principal investigators; the modes of analysis, interpretation of results and subsequent redirection of research will be by consensus, as in our cognition study.

The three main activities will be:

A. A twin-half sib study in selected countries of Europe to answer the first question (genetic involvement) and parts of the second.

B. A study in Hawaii to distinguish between modes of inheritance (question 2) and to investigate discrimination between smokers and non-smokers (question 4).

C. A study in Boulder to develop and apply animal models to address all of the questions.

Proposed Studies

A. Twin-Half Sib Study

Introduction. There are several methods of demonstrating, in reasonably unequivocal fashion, that genetic factors are involved in the development of a complex behavioral trait. One approach involves a parallel study of correlations between biological and adoptive relatives. An excellent example may be drawn from the studies of Heston (1966, 1970), Karlsson (1966) and Kety et al. (1968). These have shown a much higher incidence of schizophrenia in children separated from their schizophrenic mothers at birth than in children separated from their non-schizophrenic mothers at birth, both sets of children having received the same kind of adoptive environment. Such results have effectively terminated the argument over whether heredity

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influences susceptibility to schizophrenia and have permitted attention to turn to more detailed studies of the genetic mechanism.

Recently, formal biometrical models have been developed for the purpose of separating genetic and environmental effects in analyses of behavioral traits (Cavalli-Sforza & Feldman, 1973; Rao et al., 1974). Both models depend upon joint consideration of biological and adoptive relatives. Some of the most useful relatives are full sibs (both parents in common) separated at birth and reared apart, with placement in adoptive families at random, and half sibs (one parent in common). Other biological and adoptive situations also provide useful information for detailed genetic analyses.

There are certain problems associated with those biometrical genetic models that depend heavily on the use of adoption data. These problems include the possibility of non-random placement of children in foster homes, the possibility that heritability of smoking behavior among individuals placed in foster homes may be different from that of those raised in their natural homes, and the possibility of common prenatal environmental effects and non-additive genetic effects, both of which prevent a clear-cut separation of genetic and environmental determinants. For a full analysis of smoking behavior, information would have to be obtained on adults who had been separated early in life and on their biological and foster parents. Retrospective information on biological parents is usually obtainable in the United States only by special court order. In Europe, the laws are less restrictive, but it would still be very difficult to obtain the names of biological parents of foster children, and numerous adoption agencies would have to be approached on an

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individual basis. Finally, most agencies receive children for adoption from unwed parents; hence only one sibling is usually obtained. When two siblings are available, an attempt may be made to place them in the same home. Furthermore, their availability for placement may often result from the death of one or more of their biological, legitimate parents. Considering all of the above problems, we consider it unlikely that an adequate sample of adopted relatives could be obtained for a genetic analysis of smoking behavior.

As described earlier in the Introduction, twin studies have been used previously to implicate genetic factors in smoking behavior. While strongly suggestive of hereditary influence, the results of these studies must be considered as preliminary for several reasons. Monozygous (MZ) twins raised apart may be concordant because of their common prenatal environment. If their mother smoked during pregnancy, both fetuses may have been exposed to various effects of tobacco products at critical stages of development. If attempts were made to place MZ twins in foster homes similar to those of one or more of their biological parents, their concordance might depend on a common postnatal environment. There will also be a common postnatal environment to the extent that the twins may not have been separated until some time, perhaps several years, after birth. These problems are considerably reduced by comparison with dizygous (DZ) twins. However, from the genetic standpoint, MZ-DZ twin comparisons provide information on a type of "broad-sense" heritability. Heritability in the "narrow-sense," which is based on the average or "additive" effects of genes, is considered by most geneticists to be a more informative parameter

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in several important respects. Smoking information obtained from previous twin studies has usually been restricted to superficial questions concerning such things as whether or not a person smokes and, if so, whether or not cigarettes are involved. More detailed data on the nature of the smoking behavior and the type and amount of tobacco consumed are clearly needed.

It is possible to determine the relative magnitude of genetic control over smoking behavior in terms of the additive effects of genes (narrow-sense heritability or "h^2") by methods which do not depend on adoption data. Instead, biometrical techniques are used to study the relationship among various kinds of relatives, some of which have been raised in the same, and others in different, natural homes. We have developed a method of this sort in terms of a correlation analysis (see Appendix). Emphasis is placed on family data involving MZ and DZ twins, half sibs, and their biological and legal relatives. Two kinds of family samples are needed: Type I--common grandparent with two offspring, one of which marries twice and produces at least two half sib offspring, the other marries once and produces at least one offspring; Type II--common grandparent with two twin offspring, one of which marries once and produces at least two offspring, the other marries once and produces at least one offspring. Simultaneous equations can be generated which permit testing and refinement of the model, as well as efficient estimation of effects associated with additive genotype, assortative mating based on the additive genotype, assortative mating based on non-additive genetic and environmental residuals, three types of prenatal environments, three types of postnatal environments, and non-additive genetic and

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environmental residuals. An analysis of this sort can be used to provide a broad range of information on the interplay of genetics and environment in the control of smoking behavior.

We plan to conduct the above type of twin-half sib family study in Belgium, The Netherlands, and Sweden. The Benelux countries have the most dense, stable populations in the Western World, are small in area, and keep good census records. Location and interviewing of relatives will be easier and less expensive than in other countries of Europe and certainly much less expensive than in the United States. The major part of the study will be performed in Belgium in cooperation with Professor Pierre Feldheim, Secretary General of the Institute of Sociology at the Free University of Brussels. This cooperation is very important because it permits access to a trained staff of interviewers who can be used to obtain the detailed type of information we want for smoking behavior and related variables. We have been unable to locate a similar resource in The Netherlands. The socioeconomic diversity of Belgium will permit us to test the twin-half sib model and to estimate the various genetic and environmental parameters with a high level of statistical precision.

Due to the low divorce rate in principally Catholic Belgium, Type I samples involving half sibs may be somewhat difficult to obtain. The Netherlands will be used as a "backup" in this regard. Dr. John Streng, a behavioral geneticist at the Free University of Amsterdam and a former doctoral student at the University of Colorado, is interested in cooperating. He has informed us that he believes reasonably large numbers of half sib families can be obtained. The Dutch sample will also be useful as a check on the extent to which our Belgian

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results can be extrapolated to other ethnic groups.

The Swedish sample will provide a critically important backup to the Belgian study with respect to twin data. Professor Jan A. Böök, Director of the Institute for Medical Genetics at the University of Uppsala in Sweden, and Professor L. Friberg at the Karolinska Institute in Stockholm are prospective collaborators. Professor Friberg has a register of 25,000 twin pairs, and has been working on problems of smoking for some time. A subset of data from this Swedish twin register would provide us with various kinds of information needed in our Type II (twin) samples, including especially valuable information on the children of MZ twins. These children are genetically "half sibs." Those belonging to different male members of an MZ pair have had separate prenatal as well as postnatal environments. The Swedish data will also provide an important, additional opportunity to check on the extent to which the Belgian results can be extrapolated to other ethnic groups.

Another method for demonstrating genetic control of a behavioral trait involves its association with a known genetic variant. The variant may be a "marker" gene primarily concerned with control of a biochemical substance, such as an antigen or enzyme, or it may be a chromosome with an altered structure that is visible under a microscope. If the pattern of transmission of a genetic variant from one generation to the next can be associated with the transmission of some aspect of smoking behavior, it can be inferred that one or more genes for smoking behavior are involved.

The chance of locating any particular smoking gene by virtue of its association with a marker gene is low (Renwick, 1973). However,

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this chance will increase with the number of marker genes available for analysis, the number of different aspects of smoking behavior investigated, and the number of families included (Elston, 1973). The prospects of this approach would appear to be improved further by the existence of marker genes, such as those controlling specificity of α-1 antitrypsin and aryl hydrocarbon hydroxylase (Kimberling, personal communication; Kellerman & Shaw, 1973), which may be suspected a priori of having a relationship to smoking behavior. These genes are associated with early-onset emphysema and bronchogenic carcinoma, respectively. Models can be easily visualized in which these genes, either in homozygotes or heterozygotes, might influence smoking behavior.

The use of variant chromosomes as markers should be even more efficient. It is known from evolutionary biology that variants such as chromosomal inversions and translocations can link together large numbers of genes into complexes called "supergenes." New cytogenetic techniques are providing highly suggestive evidence that these chromosomal variants are common in human populations (Kimberling & Lubs, personal communication). If such supergene complexes contain several smoking genes of individually small effect, their transmission as a group might produce detectable effects on smoking behavior.

Whenever a smoking gene is identified by its association with a marker gene or chromosomal variant, a powerful tool becomes available for prospective studies and for use in genetic counseling. Therefore, even though this approach is much less efficient than the biometrical techniques for demonstration of genetic control of smoking,

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the benefits to be derived from it would appear to justify its use in certain situations.

We do not plan to perform the marker gene-chromosomal variant type of analysis as a part of our proposed European study on smoking behavior. However, should results of the Hawaiian and/or animal studies suggest that such an investigation is both desirable and feasible, we might wish to conduct it later, using a subset of the European families. It would be necessary to seek additional funding for such a study, possibly from NSF or NIH.

Procedures. Overall conduct of the twin-half sib study will be jointly supervised by Drs. Crumpacker, Vandenberg and Williams. They will carry out first-year pilot studies with the aid of research assistants, technicians and students. Plans for the first year are as follows:

(1) Design of smoking questionnaire and associated psychological, socioeconomic and medical questionnaires. Initial models can be obtained from previous smoking questionnaires used by the Veterans Administration, the National Research Council, the International Twin Studies, and the Addiction Research Unit of the Institute of Psychiatry in London; from several standard psychological questionnaires, such as the Eysenck personality test; and from the socioeconomic battery of the Hawaiian cognition study. These will be useful in developing instruments for our specific purposes.

(2) Definition and priority listing of information to be collected; development of self-coding, self-reproducing data forms; development of a data management system for editing, correcting, compiling and transcribing data; development of a computer data file

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for storage, retrieval, cross tabulation and administrative reporting of results; training of key data management personnel; and development of new programs and other methodology as needed.

(3) Administration of questionnaires to at least 200 people in a Denver metropolitan area "pilot" study.

(4) Multivariate analysis of the pilot data to dissect and correlate smoking behavior traits, and to isolate factors or dimensions which represent important combinations of traits. These dimensions will be the variables of prime interest for testing our model and estimating the various genetic and environmental parameters.

(5) Refinement of questionnaires and data management system on the basis of pilot study results.

(6) Arrangement of cooperative studies with key behavioral geneticists in the Benelux countries and Sweden; hiring of key foreign personnel; rental of research space; and purchase of necessary supplies and equipment.

(7) Location of foreign subjects for the study.

(8) Translation of questionnaires into French, Flemish, Dutch and Swedish, and development of a subset of questions specifically pertaining to each ethnic group.

(9) Consideration of first-year results of the Hawaii family study and the Boulder animal study.

(10) Final adjustments and refinements in questionnaires and data management system.

In the second year, collection of data in the main experiment will begin and efforts to locate subjects will continue.

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Dr. Williams will supervise the data management system through the Department of Statistics and the Computing Center at Colorado State University in Fort Collins.

From readings and discussions on smoking behavior it is apparent that the trait is extremely complex. There are many interactive components, with a broad range of etiologies. We recognize that detailed information on smoking behavior, personality, socioeconomic status and medical history must be obtained from our subjects. Because of these needs, an important portion of our budget request for the twin-half sib investigation is allocated to personal contacts with subjects. Each subject will be interviewed and tested on a personal basis.

B. Hawaii Study

The study in Hawaii has two objectives--first, investigation of the nature of genetic control of smoking behavior (question 2 above); and second, discrimination of smokers and non-smokers (question 4 above). Hawaii is chosen because of the ready availability of subjects of different ethnic groups, permitting checks of robustness of estimates in different genetic backgrounds, and because the ongoing cognition study (with 1300 families already tested) will provide access to a pool of potential subjects of known characteristics. Also, Hawaii has a Mormon population of 30,000, of whom at least 50% (perhaps 75%) are non-smokers by deference to the Mormon "word of wisdom."

Our first problem, the nature of the genetic control of smoking behavior, will be approached through analysis of family data using procedures developed by Morton and his colleagues. These include

2010050467

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path analysis (Rao, Morton & Yee, 1974) to discriminate the relative roles of polygenes and environment, and segregation analysis (Morton, Yee & Lew, 1971; Morton & MacLean, 1974) to distinguish between genetic control due to major genes and to polygenes. It should be pointed out that only by a common application of the two approaches (path analysis and segregation analysis) can the environmental, dominance, polygenic and major gene mechanisms be adequately separated. For this, both nuclear and non-nuclear families are necessary. Our family data will provide the basis for testing both major gene and polygene hypotheses. For the former, analysis will be based on segregation in biologically related families collected in our family study. For the latter, path analysis will be based on the genetic "mismatches" identified through blood and serum typing in our families. These data are extremely valuable, for if the exclusion is between child and male parent, and adoption is not admitted, then there is convincing evidence of illegitimacy. Dr. Rao considers this the most informative type of parent-offspring relationship for unravelling the correlated responses between genotype and environment, especially when a natural child is also present in the family. In our cognition study, we have approximately 5% mismatches which we are now verifying for presumed illegitimacy. In addition, we will solicit families with adopted children to supplement the adoption study described above. Very careful verification of blood typing by independent bleeding and re-analysis is needed in studies of this kind, but the information gained is worth the effort and cost involved.

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There are other approaches to the elucidation of the genetic mechanism involved in a quantitative trait. One which has attracted much interest recently is the procedure of Haseman and Elston (1972), which is based on the detection of linkage between marker genes and postulated major genes. This approach has been criticized by Robertson (1973) and by Renwick (1973) on the basis of the impracticability of measuring a sufficiently large sample to detect linkage. However, J. Gibbons in Thoday's laboratory has detected significant linkage between an enzyme locus in Drosophila and a major gene controlling approximately 1.5 bristles 15 map units away, with 200 sib pairs (Gibbons, personal communication). This finding has caused Robertson to commence similar experiments in Drosophila with other markers. The procedure is worth trying and we will do so, as we will be determining genetic markers for pedigree testing in our biological families and for the discrimination studies. Dr. Elston has agreed to accept data for analysis. His current procedures will handle sib pairs, and he is hopeful that the method will soon be applicable to larger families.

The state-of-the-art with respect to analysis of quantitative inheritance is such that more sophisticated statistical techniques are continually being developed, and this is now an issue of considerable interest to human geneticists. Notable in this field is the work of Newton Morton and his colleagues at the University of Hawaii, Robert Elston at North Carolina, and Charles Smith in Edinburgh, with all of whom we are in contact. By the time our family data are ready for analysis, we can anticipate major advances in statistical methodology from these groups and others.

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While we are mainly concerned with the identification of the nature of the genetic mechanism in smoking behavior, it should be mentioned that multivariate analysis for the separation of genetic and environmental variables (assuming polygenic inheritance) is proceeding apace. The main problem with this approach has been the inability to deal with correlated genetic and environmental responses. A recent paper by Meredith (1973) has indicated a possible solution to the problem of correlated responses. Meredith has now reached a stage beyond that reported in 1973 and has a solution to the complex equation involving an entity called B, where B is an ordinary matrix or vector of regression weights of the numerous environmental variables measured in a suitable study of quantitative variation. Dr. Meredith has recently agreed to analyze data from the first 996 families in our cognition study. If successful, the procedure or a derivation of it should be applicable to any behavioral trait, including smoking behavior, in order to derive heritability estimates relatively free from the contribution of correlated genetic and environmental responses.

The approach to the second problem in the Hawaii study, discrimination of smokers and non-smokers, consists of three parts:

(1) Is it possible to discriminate between smokers and non-smokers in a normal population without reference to smoking behavior?

(2) Is it possible to sample a population which does not smoke by choice (abstention population) and obtain the same discrimination within the population?

(3) Do the discriminated groups within the abstention population react as smokers and non-smokers with respect to tobacco-related diseases?

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Answers to the first two of these questions will be sought by investigating possible discriminating factors between smoking and non-smoking parents in our cognition study, and by comparing this normal population with a sample of an abstention population (the 30,000 Mormons in Hawaii).

A number of authors have reported differences between smokers and non-smokers. Much of the work is summarized in the report of a conference convened on St. Martin Island of the Lesser Antilles in January 1972 (Dunn, 1973). Table 1 of a chapter by Dunn lists individual traits and group characteristics which distinguish a group of smokers from a group of non-smokers, including personality traits, life-style characteristics, morphological traits and demographic characteristics. Using personality tests measuring introversion/extroversion and similar characteristics, Smith (1969) was able to classify correctly 68% of a population of 1400 college and nursing students with respect to smoking behavior disclosed later and 79% of a sample of junior high school females. A rather similar approach based on discriminant function analysis of habits of nervous tension (HNT) has been reported by Thomas (1973). She found it possible to classify correctly 70.7% of lifetime non-smokers and 64.3% of continuing smokers by applying the HNT scale to 499 former medical students 25 years after graduation. Most personality traits have a moderate heritability as measured by twin studies (Lerner, 1968), and it is not unreasonable to suppose that the discrimination was based in part on genetic attributes of the personality tests.

However, other potential discriminating factors are known. For example, there is evidence that ability to taste PTC differs between

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smokers and non-smokers, with the proportion of tasters being higher among smokers (Thomas & Cohen, 1960). In contrast to this, however, Fischer, Griffin and Kaplan (1963) found, in a study of taste thresholds for quinine, that the proportion of smokers is lower among persons sensitive to the taste of quinine. No correlation between smoking habit and sweet, sour or salty substances is known. It is also known that smokers differ from non-smokers in the relative frequency of α, β and θ waves in EEG scans. The above individual differences are either known or considered to have a genetic basis.

It is unnecessary to present an inventory of all known or reported differences between groups of smokers and non-smokers. Many are specious and not confirmed. In establishing differences between smokers and non-smokers, one may distinguish between differences due to physiological response to the act of smoking and those due to underlying genetic differences. The former category includes differences in blood chemistry, physiological response, neurological response and so on, as reported when before-smoking and after-smoking measures are compared. In the latter category are those differences which we consider to be potentially useful in discrimination--those which can be demonstrated to be genetically controlled in whole or in part. Thus, we will investigate such parameters as results on personality tests, tasting abilities, genetic polymorphisms determined in blood and saliva, individual variation in enzyme activity not influenced by the act of smoking, similar variation in measurable hormone levels, and morphological variation. The results of the animal studies to identify pharmacogenetic differences will be very relevant to this research.

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Our search will start with the 1300 male and female parents (2600 individuals) already tested in our cognition study, for whom we have blood group data, cognitive scores and some socio-economic data. These individuals will be asked to attend a session to provide information about their smoking habits, to answer a personality questionnaire, and to give a blood sample for enzyme, hormone and other biochemical analyses. The smoking questionnaire and the personality questionnaire will be standardized with those of the European twin-half sib study. The sample size will then be extended by using additional parents and mature offspring identified from the cognition study. The search for discriminating factors will be progressive, with recall of subjects as necessary to build up the test battery. This will require close contact with other active workers in the field, such as Dr. Eysenck in London and Dr. Friedman in Oakland.

The factors measured will be analyzed by discriminant function analysis to identify a smoking phenotype and a non-smoking phenotype among the subjects we test. The distribution of the frequencies, means and variances of these factors in our normal population will then be compared by appropriate statistical techniques with corresponding parameters in the Mormon population of abstainers. As far as possible, the samples of the two populations will be matched for sex, age, race and other identifiable variables.

If it proves possible to identify factors which, in conjunction, discriminate between smokers and non-smokers, and if the distribution of these factors proves to be the same in an abstaining population, then it would be reasonable to investigate presumed smokers and

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non-smokers with respect to smoking-related diseases in a population of abstainers. In addition to the Mormon group in Hawaii, the 350,000 Mormons in California and the 1.1 million Mormons in Utah would probably provide an abstaining population of sufficient size and with adequate disease records (Engstrom [1974] has reported on cancer deaths and total mortalities among Mormons in California and Utah).

If the discriminated groups within the Mormon population showed susceptibilities to smoking-related diseases paralleling those found in a non-abstaining population, then it could be concluded that genotype contributes to susceptibility to those diseases. This would confirm Fisher's (1957a) hypothesis.

Subjects and personnel. As stated previously, one of the prime advantages of conducting this study in Hawaii is the ongoing research on the genetic and environmental bases of human cognition. This research involves the collaboration of four investigators at the University of Colorado in Boulder and four investigators at the University of Hawaii, with G. C. Ashton as principal investigator. The goal of the cognition study is to test 3000 families representing different ethnic groups to determine the relative roles of genetics and environment in performance on 15 cognitive tests, subsequently reduced to four independent factors by factor analysis. Each individual in the study is evaluated by response to a psychometric test battery, and each is asked to complete an environmental questionnaire and to provide blood and saliva samples for determining a range of genetic markers.

We propose to use the parents from the cognition study and their sibs as the offspring group in our family study of smoking behavior.

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These individuals are not older than 60 years of age. From administration of a very simple smoking questionnaire during recent test sessions, we found that of 122 male adults, 40 were smokers, 35 ex-smokers and 47 non-smokers, while the corresponding figures for their wives were 31, 22 and 69. These subjects are very cooperative and willing to come back for additional testing. The parents of these adults will be contacted to provide the parental group in the proposed study of smoking behavior. Also, participation of families not represented in the cognition study will be solicited, with the main criterion being that no offspring tested should be less than 30 years of age (to ensure correct classification of smoking habit).

The Mormon population will be chosen by solicitation through the Church of Latter Day Saints, which is influential in Hawaii. Contacts with the Hawaii campus of Brigham Young University at Laie (Dr. Rathall) have already been made and cooperation promised. Mr. R. Moon, a graduate student in the Department of Genetics and a practicing Mormon, is interested in helping with the project. Mormons seem to be very interested in research which is relevant to their "word of wisdom," which advocates abstinence from smoking, drinking coffee and consuming other substances considered harmful.

In the Behavioral Biology Laboratory, we have personnel and facilities for blood and saliva typing, data handling and analysis, and other activities involved in the cognition study. The staff can be increased appropriately to handle the smoking study. An alcohol investigation under the supervision of Drs. Johnson, McClearn and Wilson is already underway using many of the same facilities. We

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also have had two years of practical experience in scheduling and handling family test sessions, and the necessary auditorium, assembly rooms, etc. are available.

Dr. G. C. Ashton, formerly Chairman of the Department of Genetics at the University of Hawaii, will be principal investigator on the proposed study. Dr. D. C. Rao, of the Population Genetics Laboratory (under the direction of Dr. N. E. Morton), will be working with both the cognition data and the tobacco data. He will apply his technique of path analysis to our non-nuclear family data and will apply Morton and MacLean's complex segregation analysis to the family data. Dr. R. C. Elston of North Carolina has offered his assistance in linkage analysis. (A former student of Dr. Ashton's, Dr. R. Go, is just concluding postdoctoral training with Dr. Elston.)

Other professional contacts of interest are with the Japan-Hawaii heart and cancer study (directed by Dr. A. Khagan), which is an ongoing study of 7000 Japanese males (and their spouses) born between 1900 and 1919. Information from this study will be useful, since smoking habits are identified as well as disease outcome. Additionally, the professional assistance and criticism of colleagues at the University of Hawaii (Dr. Morton, population genetics; Dr. C. S. Chung, public health; Dr. M. P. Mi, statistical genetics; Drs. R. Cattell and R. Johnson, quantitative and developmental psychology; and others) are available when requested.

C. Animal Study

The purpose of this research program will be to utilize animal models to investigate the genetic basis of individual differences in

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tobacco-related behavior and the pharmacological and neurophysiological mechanisms underlying those differences.

The virtue of animal models is, of course, that they permit certain types of observations that cannot be made on human beings. Laboratory animals can be mated according to schedule, their exposure to nicotine can be strictly controlled, tissue samples can be obtained for pharmacological analysis, and electrodes can be placed in the nervous system. A key issue in animal research is ascertaining the relevance of the animal model to the human condition that is the ultimate object of interest. Either extreme view, that animal data can be immediately applied to the human situation or that animal data can have no relevance whatsoever, would seem to be inappropriate. A balanced view regards the animal research as generative of hypotheses to be tested at the human level and as conditional tests of hypotheses generated by human results but not testable on human beings.

The rationale for use of animal models is based upon consideration of the evolutionary process. The adequacy of, say, a mouse model of tobacco-related behavior will depend upon the extent to which the mammalian heritage common to mouse and man is relevant to that behavior. There is no a priori method of determining the usefulness of this type of research; it is strictly an empirical question.

Animal models of behavioral processes are sometimes criticized because they are not complete in all details. Complete models would be desirable, of course, but it seems probable that specific dimensions of a complex problem, such as smoking behavior, can be illuminated by partial models. It is likely that greater ultimate progress will be made by using several partial models in parallel

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than by awaiting the development of the perfect model (which, in fact, might never be attained).

While the utility of mouse research on the behavioral pharmacogenetics of tobacco-related behavior cannot be guaranteed in advance, the general success of animal models in biomedical research warrants a sizable investment. The modest amount of evidence available to date on genetic differences among animals in response to nicotine (Bovet-Nitti, 1969; Bovet, Bovet-Nitti & Oliverio, 1967; Garg, 1969b) justifies optimism about the outcome.

Several different approaches will be integrated in this research program. For example, information from studies on strain differences in effects of nicotine on open-field activity would suggest pharmacological and neurophysiological investigations; the results of these might stimulate further exploration at the behavioral level and/or further genetic manipulation; and so on. It is thus not possible to specify with any accuracy the nature of the "second wave" of research. However, in spite of the interdependence of the different approaches, specific experiments can be initiated in each simultaneously for the "first wave" of research. The nature of these initial projects is indicated below. For convenience of discussion, genetic, behavioral and mechanism research techniques will be described separately. It should be emphasized, however, that these approaches will be intimately intertwined in the actual conduct of the program. In particular, genetic controls or manipulations will be a feature of every experiment.

Genetic techniques. In studying the genetic basis of tobacco-related behavior, use will be made of inbred mouse strains, a

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genetically heterogeneous population, selectively bred lines and strains segregating for single loci. The strengths and weaknesses of these groups have been discussed by McClearn (1972) in reference to alcohol research. That paper is incorporated in this proposal as Appendix A.

Inbred strains. Strains currently available in the specific-pathogen-free laboratory of the Institute for Behavioral Genetics include C57BL/6, DBA/2, C3H/2, BALB/c and A. The strain screening to be undertaken early in the research program will supplement these strains with others obtained from the Jackson Laboratory. These latter strains will be housed and tested in conventional laboratory space; if any of them display interesting characteristics relevant to the proposed research, they will be introduced to the specific-pathogen-free facility by caesarean procedures.

Heterogeneous stock. A genetically heterogeneous stock is particularly appropriate for assessing hypothesized phenotypic correlations, for use in family resemblance studies on heritability and genetic correlations, and as a base population for selective breeding studies. The origins of the heterogeneous stock (HS) maintained at the Institute for Behavioral Genetics, and some considerations of their research utility, have been discussed by McClearn, Wilson and Meredith (1970). That discussion is included here as Appendix B.

Selectively bred lines. Selective breeding permits the deliberate development of animal lines that differ with respect to some specific characteristic of research interest. In principle, successful selective breeding requires only a non-zero heritability.

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In practice, extremely low heritability might make a selective breeding program too inefficient, although research in other areas has shown the utility of selection procedures when h^2 is about 0.20. For example, long-sleep and short-sleep lines developed and maintained at the Institute show, respectively, high and low sensitivity to alcohol as measured by a "sleep-time" test. After 15 generations, these groups were practically non-overlapping with respect to the trait under selection even though realized heritability was calculated to be 0.18.

Selective breeding is an expensive procedure in terms of personnel, time and space. Therefore, it should only be employed to generate animals differing on key, crucial dimensions. Because the procedures should not be changed once selection has begun, the methods of measuring the phenotype should have been well standardized before beginning the program. On the other hand, because of the basic limitation on the pace of a selection study imposed by the generation interval of the experimental animals, it is important to begin as soon as possible. Much of our early research will therefore be oriented toward resolving issues preparatory to initiating one or more selection programs. Although the precise phenotype(s) to be selected must be identified from results of this preliminary research, it seems likely that the general domain will be appetitive behavior to nicotine, measures of sensitivity to the effects of administered nicotine, or measures of acquisition of tolerance to or dependence on the effects of nicotine.

The foundation stock for all selection studies will be the HS mice described above. Generation zero will be composed of 50 HS

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females and 50 HS males. Mass selection will be employed. Approximately 10 males and 10 females will be selected in each direction. Litter sizes in the HS are such that this number of matings will provide about 100 testable offspring in each line each generation.

In addition to the selective breeding for specific tobacco-related behaviors, available lines selectively bred for other behaviors can also be examined. For example, the human literature suggests a differential response to nicotine by people with different personalities. Perhaps the nearest analogue to a personality measure in mice is activity level, so animals differing in this dimension would be a useful model for research on this topic. The Institute maintains two activity selection studies at the present time. DeFries (DeFries, Hegmann & Halcomb, 1974) has selected bidirectionally for open-field activity with replicate high and low lines and controls. These selected lines are widely separated. McClearn (unpublished) has selected for wheel activity. These lines have not diverged to the extent of the DeFries open-field lines, but they do display a significant mean difference.

Single-gene research. Identification of a single locus that influences tobacco-related behavior would provide extremely valuable research material, particularly for the study of pharmacological and neurophysiological mechanisms. The ideal situation is one in which a mutation has occurred on an inbred background. Nearly as useful are inbred strains in which a mutant allele has been introduced by successive backcrossing.

The a priori probability of a particular single locus influencing tobacco-related behavior is not high. However, a

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considerable number of loci are now available on inbred strain backgrounds. Although screening these loci for relevant effects would be frankly speculative, the value of a positive finding would be so great as to justify a substantial investment.

Behavioral techniques. Various behavioral techniques will be employed to assess appetitive or seeking behavior for nicotine or other tobacco products and to determine the effects of nicotine upon behavior. In addition, behavioral situations will be used as independent variables in exploring genotype-environment interactions with respect to both appetitive behavior and nicotine effects.

Appetitive behavior. Assessment of preference for, or choice of, nicotine or other tobacco products will be approached in several ways. The most efficient method will be to offer nicotine to the animals in aqueous solution. A two-bottle choice situation, such as that used extensively at the Institute in alcohol studies, might be most useful. Some work currently in progress has demonstrated the feasibility of this approach, and has shown substantial individual differences among mice in preference for the nicotine solution. However, other techniques involving presentation of multiple concentrations or a single-bottle procedure will also be evaluated for suitability for nicotine research.

An alternative choice situation would be to investigate preference for tobacco smoke. A situation involving smoke inhalation has many advantages as a model for human smoking, but there are many acknowledged difficulties. We shall attempt to design a system to interface with the smoke generators developed by other investigators for use in choice situations with mice.

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Yet another approach is to utilize operant procedures in which the animal's response can administer a metered dose of nicotine via an indwelling catheter. Permanently implanted intravenous catheters were first employed in this way in freely moving animals by Sharpless (1959, 1961) to study the potential reinforcing effects of sympathomimetic amines. The method has since become a standard technique for investigating self-administration of drugs. Deneau and Inoki (1967) found that monkeys would self-administer nicotine solutions (as well as most other drugs abused by man) through indwelling intravenous catheters. There appeared to be a maximally effective dose for each monkey above which the animal refused to self-administer nicotine. More recently, Clark (1969) employed the self-administration technique for studying nicotine intake by rats. We shall use Clark's procedure to carry out initial experiments on commercially available strains of rats. After the animals have learned to self-administer nicotine, the reinforcement schedules and dose regimens will be manipulated in an effort to determine whether different strains show preferences for characteristic dose regimens.

Activity. As mentioned above, activity level might be regarded as a personality variable in mice. Thus, in view of the demonstrated personality correlates of smoking behavior in man, this is a particularly attractive area of study. However, mouse activity is a complex of behaviors--not a single, measurable trait. Using a battery of activity measuring devices, the present investigators have demonstrated the existence of several independent dimensions of activity and have shown that these dimensions are influenced by different endocrine systems.

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The full activity battery is available for the proposed research. It consists of eight apparatus situations, each of which yields several measures. More details of the battery are provided in Appendix B.

Social behavior. Much of human smoking behavior occurs in a social context. Furthermore, many of the personality variables putatively related to smoking behavior involve or imply social responses. For these reasons, the study of the influence of nicotine on mouse social behavior, and of the influence of social settings on tobacco-related behavior, is particularly appropriate.

Three general aspects of social behavior will be studied: gregariousness, sexual behavior, and aggression. Procedures for quantitative assessment of gregarious (or aggregative) behavior in mice have recently been developed in our laboratories.

Sexual behavior is not a single, global trait, but it can be broken down into a large number of discrete components. This has been done in studies on rats, guinea pigs, mice, and several other species. By capitalizing upon the existence of moderately complex scoring systems developed by other investigators, it is possible to examine changes in discrete components of the sexual behavior pattern as a function of antecedent conditions, including the administration of nicotine.

Two main research tactics will be employed. The first is to study the effects, if any, of nicotine on the reproductive performance of pairs of mice housed together. Some of the variables to be examined will be: (1) mean time to parturition, after pairing; (2) mean litter size at birth; (3) mean offspring weight at birth;

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(4) mean litter size at weaning; (5) mean offspring weight at weaning; and (6) reproductive performance of offspring whose parents were treated with nicotine. Any differences between treated and control subjects will then be the focus of more detailed studies. For instance, a difference in mean litter size at birth could be studied by determining whether the number of eggs ovulated had been reduced by the drug or if, perhaps, a normal number of zygotes had been formed, but some were resorbed during pregnancy. A difference in mean litter size at weaning (assuming no difference in number born) could be studied by examining the adequacy of maternal care.

The second main tactic will be to investigate the effects of nicotine on sexual behavior patterns of both male and female mice. It should be possible to relate any changes to neurological and pharmacological differences found in other phases of this research program. Changes in sexual behavior should also be relatable to reproductive differences demonstrated by the procedures described above. In both cases, stable correlations should furnish specific and testable hypotheses for studying the effects of smoking on human sexual behavior and fertility.

Several methods for evaluating aggressive behavior have been used in our laboratories. Paired encounters of brief duration between male mice in testing arenas, a classic technique, have been employed by Klein, Howard and DeFries (1970) to demonstrate the strong interaction of genetic and environmental variables. St. John and Corning (1973) have explored techniques for eliciting female aggression, which does not occur in the classical situations, and have shown a marked hereditary influence on the intensity of such aggressive behavior.

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Also, DeFries and McClearn (1972) have developed a long-term social situation in which the full range of mouse social interaction can be studied. We are now adapting an infra-red television system to enable us to time-sample behavior in this and other situations throughout the night (when nocturnal animals, such as mice, are most active).

Results of studying these aspects of social behavior can be used in correlational studies to assess their association with appetitive behavior for nicotine or other tobacco products. They will also serve as dependent variables in assessing the effects of administered nicotine. Finally, the social situations can be imposed as independent variables to determine their effectiveness in altering tobacco-related behavior. For example, one situation that has been pilot-tested in alcohol research imposes stress through placing male mice together in different, unfamiliar groups each day. This type of stress, resulting from intensive social interaction and aggression, would appear to be more similar to human stress situations than that produced by administering electric shock or by other "artificial" means which are commonly used in animal research. It is quite possible that such stress may influence tobacco-related behavior.

Learning and memory. The initial experimental approach in this behavioral domain will be to study the effects of chronic nicotine exposure on memory processes of inbred strains of mice.

During the past several years, precise techniques have been developed in our laboratories for quantifying the development and time course of short-term memory (STM) and for ascertaining the strength of long-term memory (LTM) (Alpern & Marriott, 1972a,b, 1973; Marriott & Alpern, 1973). In addition, evidence has been obtained to indicate

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that cholinergic mechanisms are necessary for the proper functioning of STM. Specifically, decay of STM can be markedly accelerated by scopolamine and notably slowed by physostigmine (Alpern & Marriott, 1973). It has also been demonstrated that the long-term store of memory can be quantified and manipulated pharmacologically (Alpern & Crabbe, 1972; Crabbe, 1973; Crabbe & Alpern, 1973; Stripling & Alpern, 1974). Acute administration of stimulants such as strychnine and pentylenetetrazol have a facilitatory effect on the long-term memory store, whereas other stimulants (such as d-amphetamine, nicotine and caffeine) have a disrupting effect over a wide range of dosages.

We plan to employ these procedures in the proposed program to study the chronic effects of nicotine and nicotine alkaloids on the various stages of memory. Initially, our strategy will be to expose mice of inbred strains to several concentrations of nicotine in vitamin-fortified Metrecal for various periods, extending to 15 months, and to evaluate effects on STM, LTM and the long-term store of memory. Appropriate controls will be included to ascertain the effects of liquid diet, possible malnutrition, etc. Advantages of this experimental design are that, in addition to measuring the effects of prolonged ingestion of nicotine on the various stages of memory, we can also investigate withdrawal patterns as a function of nicotine exposure and effects of age upon memory systems.

Subsequent research in this area will depend upon results of the initial work. For example, if memory deficits are obtained in the above study, an attempt will be made to reverse these deficits with drugs. If STM impairment is found, physostigmine will be used

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(see Alpern & Marriott, 1973). If LTM or long-term store deficits are observed, strychnine or pentylenetetrazol will be employed (see Alpern & Crabbe, 1972; Crabbe & Alpern, 1973; McGaugh & Petrinovich, 1965).

The REM stage of sleep has been consistently implicated in memory processes (Fishbein, 1969; Hartmann & Stern, 1972; Joy & Prinz, 1969; Miller et al., 1971; Pagel et al., 1973; Pearlman & Becker, 1973). We propose to implant cortical electrodes in mice, a procedure whereby REM sleep can be distinguished from the non-REM stages of sleep. Sleep patterns will be assessed prior to and after nicotine withdrawal. We shall ascertain the effects of chronic administration of various dosages of nicotine upon sleep patterns and correlate this information with memory effects.

In other animals, electrodes will be implanted in the visual cortex and lateral geniculate, as well as in the auditory cortex and medial geniculate, one week prior to the time when the animals become 3, 6, 12 or 15 months old. Visual and auditory stimuli will be presented at these ages, and evoked potentials will be recorded. Latencies of onset of electrical responses in the thalamus and cortex can then be used as indicators of neural efficiency. Effects of chronic administration of several dosages of nicotine upon neural response latencies, and latency measures obtained before and after withdrawal, will be the data of interest.

Mechanism research. This approach involves the study of pharmacological and neurophysiological mechanisms through which genetic influences are mediated. Although the design of most of these studies will depend upon the outcomes of prior behavioral investigations,

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certain specific projects can be undertaken immediately.

Pharmacological studies of nicotine tolerance and dependence. Physical dependence has classically been considered to be related to the development of tolerance. There are two different possible sources of tolerance: Pharmacokinetic tolerance results from an alteration in one of the parameters which control the concentration of the drug in the body. These parameters include absorption, metabolism, distribution and excretion. Pharmacokinetic tolerance is most often metabolic in origin, in that the rate of metabolic inactivation of the drug is increased as a consequence of chronic administration. Tissue tolerance, in the case of a drug such as nicotine, refers to a decreased response of the nervous system to the actions of the drug. Of these two possible sources, it seems most likely that dependence is related to tissue rather than to pharmacokinetic tolerance.

Thus, our initial strategy will be to (1) develop fully a convenient and reliable method of assessing the actions of nicotine, (2) compare several inbred strains of mice with regard to sensitivity to the effects of nicotine, and (3) compare the rates of metabolism of nicotine in these strains. These data will provide information as to whether strain differences in the acute effects of nicotine exist and if the source of the effects is metabolic or may involve tissue tolerance.

Because nicotine is metabolized rapidly by the liver, it is virtually impossible to maintain consistent nicotine levels in various tissues by oral administration. Therefore, for studies requiring the chronic administration of nicotine, such as the development of central and peripheral tissue tolerance and physical dependence, various methods

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of administration of nicotine will be examined.

The first study will involve the administration of nicotine (free alkaloid) in inert oil bases, e.g., peanut oil, subcutaneously and intramuscularly. Blood and tissue levels of ^{14}C-nicotine will be determined at various times following administration. These levels will be compared with levels achieved by intraperitoneal injections. It may also be possible to develop resin-impregnated pellets containing nicotine, to be administered subcutaneously, which would slowly release the nicotine. After obtaining a suitable procedure to provide a continuous tissue level of nicotine, this procedure will be adopted in studies of the development of both pharmacokinetic and tissue tolerances.

In the next phase of the research, animals will be treated chronically with nicotine or tobacco smoke, and the rate of development of tolerance to nicotine effects will be assessed. Once again, rates of metabolism will be measured in various strains as they are being treated. Thus, the source of tolerance can be estimated as it develops.

Our initial efforts will involve intravenous injections of ^{14}C-nicotine solutions following both acute and chronic nicotine treatments in various inbred strains of mice. At various time intervals blood samples will be taken from each mouse, serially, by means of a retro-orbital sinus bleeding technique which we have developed. Nicotine present in the blood and other tissues will be separated from its metabolic products, and the amount of nicotine will be determined by liquid scintillation counting. In addition to determining the rate of disappearance of nicotine from the blood, the

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metabolism in vitro of nicotine by liver microsomal enzyme preparations from the various strains will be examined. Possible differences in tissue distributions of nicotine will be similarly determined at various time intervals following intravenous injections of radiolabeled nicotine. These studies will determine nicotine levels in various areas of the brain, as well as in some peripheral tissues.

Data obtained on rates of nicotine metabolism and differences in nicotine concentrations in tissues after nicotine administration in various inbred strains will be compared with data showing inbred strain differences in the effects of nicotine on neurophysiological parameters such as theta rhythms and sensory evoked potentials, to be described later.

The inbred strains of mice will also be examined for differences in other pharmacological responses to nicotine. These studies will include comparison of LD50 values for nicotine. Resulting strain differences will be evaluated on the basis of whether they arise through differences in rates of nicotine metabolism or tissue sensitivity.

Additionally, studies will be carried out which will determine the sensitivity to nicotine of gut strips and other smooth muscle preparations obtained from the various inbred strains. Similarly, differences in the effects on other physiological responses, such as heart rate and respiratory rate, will be determined. Taken together, these studies will provide information as to the magnitude of tolerance as well as its source.

Once strains of mice have been identified which differ in tolerance to the effects of nicotine, or in the rate at which tolerance

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develops, studies can be carried out to ascertain the relationship between tolerance and the development of physical dependence. These studies will provide information as to the roles of metabolic and tissue tolerance in the development of dependence upon nicotine.

The hypothesis that tolerance is related to dependence can also be approached by selective breeding. Selection pressure will be exerted to develop lines of mice from the HS background which differ in tolerance to nicotine. As these lines are developed, the animals will be tested to determine the source of the difference in tolerance.

Information obtained by using animal models to investigate nicotine tolerance and dependence will be interesting in itself. It may also allow us to predict whether humans may differ for a genetic reason with regard to the likelihood of developing dependence upon nicotine.

Pharmacological and neurophysiological correlates of self-administration. Should strain differences in self-administration of nicotine via intravenous catheter be found, they will be exploited in an attempt to determine the pharmacological actions associated with the dose preferred by each strain. In this way, it may be possible to narrow the range of pharmacological actions of nicotine which are responsible for self-administration, i.e., to determine the nicotine-induced central nervous system state which the animal is endeavoring to maintain. It might be found, for example, that (1) the preferred dose is different in different strains but in every case is just adequate to produce a marked hippocampal theta rhythm, and that (2) the sensitivity of the latter index covaries with the preferred

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dose, while other indices of nicotine's central actions vary independently. Such a finding would suggest that the animal was attempting to regulate its theta activity by nicotine self-administration. In that event, sensitivity of the theta rhythm generator to nicotine would be a predictor of nicotine preference behavior.

Genetic variation in central nervous responses to nicotine. Research on physiological variations among strains requires screening techniques which can be utilized with large numbers of animals. This requirement precludes highly analytic techniques (e.g., microelectrode recording) which introduce large sampling errors, except perhaps to test specific hypotheses after strain differences have been established. It is necessary to rely on indices of the central nervous reactions to nicotine that are less easily interpreted but more easily recorded. For this purpose, we have provisionally selected certain physiological indices of nicotine action for assessing the sensitivity of various strains to the drug.

When nicotine is injected intraperitoneally (i.p.), it is taken up largely by the portal circulation and undergoes degradation by the liver so that the peak brain concentration is affected by the rate of metabolism in the liver. The LD_{50} to i.p. injections is affected by inducing drug-metabolizing enzymes in the liver; the LD_{50} to intravenous (i.v.) injections is not so affected (Stalhandske, 1970). To assess central nervous sensitivity to nicotine, it is therefore necessary to use i.v. injections and to measure the immediate effect of the injection before there is time for significant hepatic degradation of nicotine to occur. We shall use both methods of administration, i.p. for initial screening and then i.v. injections

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to exclude differences in rate of metabolism.

Extensor convulsions and lethality. The relative importance of central nervous and peripheral effects in the lethal effects of nicotine has been uncertain. Recently, however, Aceto and associates (1969) observed that ganglionic blocking agents blocked nicotine-induced extensor convulsions and reduced mortality when the drugs were injected directly into the ventricles of the brain. TEA, atropine and morphine were without effect. This would suggest that extensor convulsions and lethality in mice are at least partly due to a direct effect of nicotine on nicotinic receptors in the brain. The genetic determinants of seizure susceptibility, and its biochemical and behavioral correlates, have been an important research area in our laboratories for a decade. A great deal is known about the genetic variation in susceptibility to various kinds of seizures in mice. To our knowledge, however, there has been little study of the genetic determinants of nicotine-induced seizure susceptibility.

Cortical desynchronization. EEG's will be recorded, using implanted electrodes and computer-aided analysis, to investigate differences in the sensitivity of various strains to nicotine, DMPP, and other cholinergic agonists and antagonists. Domino and associates have shown that there are both muscarinic and nicotinic modes of activating the EEG (Domino et al., 1968). We have developed simple techniques for electrode implantation in mice, requiring 5-15 minutes per animal. These techniques will permit us to obtain repeated measures under various drug conditions. If interesting strain differences emerge, more analytic experiments employing quarternary agents, or agents which mimic one or another of the peripheral actions

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of nicotine without affecting the EEG, will be conducted. We have had experience in using such techniques to dissociate the central and peripheral actions of nicotine on the spinal cord (Goldfarb, 1971; Goldfarb & Sharpless, 1971).

Hippocampal theta rhythms. One of the more interesting effects of nicotine is the induction of theta rhythms in the hippocampus. Of various grossly recorded brain waves, the theta rhythm bears the most intriguing relations to behavior. Vanderwolf (1969) found that theta rhythms in the hippocampus precede and accompany gross voluntary types of movement such as walking, rearing, jumping, etc. The onset of a shock avoidance response is preceded by theta, which increases in frequency until just before the response occurs. Theta is not associated with behavioral immobility or automatic movement patterns. The frequency of the nicotine-induced theta rhythm increases with dose. Domino and associates (1968) noted that theta rhythms, unlike nicotine-induced EEG desynchronization, can be blocked by atropine. Intraventricular hemicholinum-3 also abolishes spontaneous theta rhythms but does not prevent EEG desynchronization. These findings suggest that, while both responses involve a cholinergic link, they are pharmacologically distinct.

Theta rhythms have not been extensively studied in the mouse brain. The demonstrated relations between theta and behavior suggest that the sensitivity of this mechanism to nicotine might be related to rearing behavior in rodents. Nicotine induces rearing in certain strains of rats, but there are marked strain differences (Garg, 1969a,b). In some strains, nicotine actually reduces the frequency of rearing (Keenan & Johnson, 1972). Moreover, the effect

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changes with repeated administration, and there is evidence of rebound changes (abstinence effects) when nicotine is withdrawn after repeated administration.

The above findings suggest that it would be of considerable interest to seek differences among mouse strains in the theta generator, particularly in its response to nicotine and other cholinergic agents.

Sensory processing and evoked potentials. There is scattered evidence implicating nicotinic action in structures involved in the transmission of sensory information in the primary sensory pathways to the cortex (Sabelli & Giardina, 1972; Hall et al., 1973). Other evidence suggests that a nicotinic relay forms part of an intracortical inhibitory system in the sensori-motor cortex (Phillis & York, 1968; Stevens, 1973).

Although it is difficult to obtain information about the organization of the underlying neural networks from studies of grossly recorded evoked potentials, it is to be expected that changes in these potentials would occur when such mechanisms are exposed to nicotine and related agents. Employing the implanted electrode technique developed for mice, it would be practical to screen animals in fairly large numbers for strain differences in the effects of nicotine and other cholinergic agents on sensory evoked potentials. The method can be adapted to recording visual and somatosensory, as well as auditory, evoked potentials from mice.

Genetic variation in susceptibility to tolerance and dependence. In the studies employing electrophysiological indices to measure sensitivity to nicotine injected intravenously, the development of tolerance based upon decrease in brain sensitivity

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can be investigated. It is entirely possible that the different indices of nicotinic action will reveal differential tolerance. For example, the optimum dose for eliciting theta may shift with repeated exposure to the drug, while that for affecting sensory evoked potentials may remain relatively constant. Variation in the rate of development of tolerance for different functions is well established for drugs such as barbiturates, where tolerance to the soporific effects of the drugs may develop with little or no change in the respiratory depressant effects of the drugs. This raises the possibility of studying genetic variation in the development of tolerance across functions as well as across strains. Tolerance to nicotine has been observed in several behavioral studies (Keenan & Johnson, 1972; Morrison & Stephenson, 1972); however, in most instances, the nicotine was administered i.p. and the effect of more rapid metabolism would have been confounded with possible changes in the central nervous response.

The methods described above, employing implanted electrodes and i.v. injections, will permit systematic investigations of the rate of development of tolerance and of rebound changes in various strains of mice.

Genetic variation at the cellular level--cloning for nicotine responses in neuroblastoma. Neurons of the autonomic ganglia are, of course, extremely sensitive to nicotine. A tumor derived from mouse sympathetic ganglia cultured some years ago has provided biologists with a rich source of genetically controlled cells having some of the properties of neurons. The neuroblastoma divide in vitro and can thus be cloned. The tissue has now gone through many generations and numerous clones are available, some of which have been

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subjected to extensive neurophysiological and biochemical investigations. Under certain conditions, exposed to ACh or deprived of serum, the neuroblastoma differentiate into neuron-like cells having different properties, depending on the cell line. We have spent some time investigating the electrophysiological properties (membrane potentials, passive electrical properties of the membrane, rectification, ability to produce action potentials during passage of intracellular current, and rate of accommodation) of certain clones. The preparation is convenient for neuropharmacological studies, because electrodes can be inserted into the cells under direct visual control and substances can be introduced into the extracellular medium while recording.

Furthermore, the cells can be cloned for specific responses to tobacco alkaloids, which is the most efficient possible way of producing genetic variations in the response to such substances. The resultant clones would then be investigated for the electrophysiological and biochemical properties associated with various models of responding to the alkaloids. The preparation permits study not only of acute effects of tobacco alkaloids on genetically distinct material, but also of effects of chronic exposure on cells of different lines.

Animal program investigators and facilities. Drs. Herbert Alpern, Allan Collins, V. Gene Erwin, Philip Groves, Gerald McClearn, Kurt Schlesinger, Seth Sharpless and James Wilson will contribute to the animal research program. The interdisciplinary approach proposed here will bring to bear various methodologies and techniques of electrophysiology, pharmacology-biochemistry, behavioral science and genetics on the common problem area. The principal methods to be employed,

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and the investigators who will be primarily responsible for them, are as follows:

Electrophysiology. (1) Development and operation of a computer-assisted system for measuring EEG, theta rhythms and evoked potentials in mice, employing both acute and chronic (indwelling electrode) techniques, suitable for screening mice for strain differences in the CNS reaction to nicotine. [Sharpless] (2) Extracellular recording from single nerve cells in mice. This technique is not suitable for screening large numbers of animals, but it provides a highly analytic tool for testing specific hypotheses. [Alpern & Groves] (3) Intracellular recording from neuroblastoma from mouse sympathetic ganglia. This technique is to be used in the work on cloned neuroblastoma grown in tissue culture. [Sharpless]

Pharmacology-biochemistry. (1) Development of chronic administration techniques. [Collins & Erwin] (2) Assessment of biogenic amines and their synthetic and degradative enzymes in brain. [Collins, Erwin & Schlesinger] (3) Measurement of tissue levels of nicotine and tobacco alkaloids. [Collins & Erwin] (4) Measurement of activity of hepatic nicotine metabolizing enzymes. [Collins & Erwin] (5) Assessment of non-CNS actions of nicotine, i.e., effects on gut movement, heart rate, etc. [Collins & Erwin]

Behavior. (1) Assessment of social behavior (aggressive, sexual, gregarious). [McClearn, Schlesinger & Wilson] (2) Assessment of locomotor activity. [McClearn & Wilson] (3) Assessment of seizure susceptibility. [Schlesinger] (4) Assessment of learning performance and memory parameters. [Alpern & Schlesinger] (5) Development of measures of appetitive behavior to tobacco. [McClearn & Sharpless]

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Genetics. Genetic techniques will be employed by all investigators. Special responsibility for classical genetic analyses (using inbred strains and derived generations), estimating genetic parameters in genetically heterogeneous animals, establishing selective breeding procedures, and single locus analyses will be assumed by McClearn and Wilson.

As mentioned above, the animal researches are expected to become increasingly interdependent as the program continues. Each area of research will begin with projects that do not require prior input from the other areas; even within this "first wave" of studies, however, information will emerge that will make possible the interweaving of the electrophysiological, pharmacological-biochemical, and behavioral approaches. The genetic perspective will be utilized throughout, of course, by use of appropriate strains or lines.

The "first wave" studies, which can be initiated immediately, will include the following technique developments: (1) method of chronic administration of nicotine, (2) computer-assisted system for electrophysiological recording in mice, (3) method of self-administration. Concurrently, there can be studies concerning effects of acute administration on: (4) activity, (5) sexual behavior, (6) seizure susceptibility, (7) development of tolerance, (8) development of dependence, (9) tissue levels of nicotine and other tobacco alkaloids, (10) metabolism of nicotine.

While awaiting development of inhalation and indwelling catheter techniques, research on self-administration can proceed by screening inbred strains with respect to: (11) consumption of nicotine in drinking choice situations.

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When the computer-assisted electrophysiological techniques have been refined, assessment can be made of: (12) effects of acute nicotine doses on EEG, theta rhythms and evoked potentials.

After development of a useful mode of chronic administration, it will be possible to study effects of chronic dosages of nicotine on: (13) memory processes, (14) tolerance, (15) dependence.

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APPENDIX

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APPENDIX

Twin-Half Sib Analysis

I. Introduction

In this section we set out the mathematical model for describing correlations of smoking behavior between relatives as functions of genetic and environmental effects and the statistical procedures for testing the model and for estimating magnitudes of the effects. The principal purpose of this development is to provide an objective basis for choosing relationships to be studied and for determining sample sizes for each.

II. Model for Quantitative Inheritance

All discussion will be restricted for the sake of mathematical simplicity to a phenotype under control of several diallelic loci. Thus, the genotypes of individuals randomly sampled in a well-defined population can be represented by a (ℓ x 1) vector, $\underline{G}$, where the $\underline{i}$th position denotes the allelic constitution of the $\underline{i}$th locus, viz. -1 for --, 0 for -+, and 1 for ++.

The random variable phenotype of individuals will be denoted by P and will be taken as a function of $\underline{G}$ and an (m x 1) vector, $\underline{E}$, of environmental variates, i.e., $P = P(\underline{G},\underline{E})$. Unlike $\underline{G}$, the random variable, $\underline{E}$, has neither a direct nor operational definition. Both the functional form of P and the existence of $\underline{E}$ constitute assumptions made to simplify the ensuing mathematical development. However, the function is not in any way restricted, so genotype x environment ($\underline{E}$) interaction is not excluded, and the statistical relationship between $\underline{G}$ and $\underline{E}$ is arbitrary so that non-zero genotype-environment covariances and partial genetic control of the environment are admissable.

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Genetic and environmental scores, say G and E, are random variables defined in the standard way, viz.

$$G = \xi_{P|\underline{G}}[P - \xi(P)] \quad \text{and} \quad E = P - \xi(P) - G \ . \qquad [1]$$

Notice that there is no expression of genotype x environment interaction in these definitions. To incorporate this it would be necessary to index P by a random variable, F, and to define the conditional scores,

$$G(F) = \xi_{P|\underline{G},F}[P - \xi_{P|F}(P)] \quad \text{and} \quad E(F) = P - \xi_{P|F}(P) - G(F) \ . \qquad [2]$$

Only in situations where clear characterization of the environment is possible (e.g., child reared in natural home versus reared in foster home) is this refinement meaningful, but then analyses within subpopulations using [1] are more useful.

It is easily seen from the definitions in [1] that

$$\xi(G) = \xi_{\underline{G}}(G) = 0 \ , \qquad [3]$$

$$\xi(E) = \xi_{P|\underline{G}}(E) = 0 \ , \qquad [4]$$

$$\xi(GE) = \xi_{\underline{G}}[G\xi_{P|\underline{G}}(E)] = 0 \ . \qquad [5]$$

This shows clearly that genetic and environmental scores do not correlate, even though elements of $\underline{G}$ and $\underline{E}$ may. In fact, the effects of covariances between $\underline{G}$ and $\underline{E}$ on the analysis of correlations in P are very complicated and very subtle, as will be illustrated in subsequent discussions of modeling consanguineous relationships with path diagrams.

A result of [5] is that the phenotypic variance is the sum of the genetic and environmental variances, i.e., $v_P = v_G + v_E$.

The additive genetic score associated with the genotype, $\underline{G}$, is defined to be the best fitting linear combination of the elements of $\underline{G} - \xi(\underline{G})$ to G. This is

$$G_a = \xi(G\underline{G}')C_{\underline{G}}^{-1}[\underline{G} - \xi(\underline{G})] \ , \qquad [6]$$

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where $C_{\underline{G}}$ is the matrix of variances and covariances of the elements of $\underline{G}$. The matrix is diagonal if loci determining $\underline{G}$ are in linkage equilibrium. The additive genetic variance is

$$v_{G_a} = \xi(G\underline{G}')C_{\underline{G}}^{-1}\xi(\underline{G}G) \ , \qquad [7]$$

and reduces to $\sum_{k=1}^{q}\mathrm{cov}^2(GG_k)/\mathrm{var}(G_k)$ for the diagonal special case. The additive score does not correlate with E because

$$\xi(G_aE) = \xi(G\underline{G}')C_{\underline{G}}^{-1}\xi(\underline{G}E), \quad \xi(\underline{G}E) = \xi_{\underline{G}}[\underline{G}\xi_{P|\underline{G}}(E)] \quad \text{and} \quad \xi_{P|\underline{G}}(E) = 0 \ . \qquad [8]$$

The nonadditive genetic score is the genetic residual

$$G_{na} = G - G_a \qquad [9]$$

and does not correlate with G_a because of the manner in which the latter is defined. The correlation of G_{na} with E is also zero, because $\xi(G_{na}E) = \xi(GE - G_aE) = \xi(GE) - \xi(G_aE)$. The nonadditive genetic variance is $v_{G_{na}} = v_G - v_{G_a}$ as a result of the zero correlation between G_a and G_{na}.

In summary, the phenotype may be expressed in two ways, either

$$P = P(\underline{G},\underline{E})$$

or

$$P - \xi(P) = G_a + G_{na} + E \ .$$

In the former, the existence of a functional form, $P(\cdot,\cdot)$, and a vector, $\underline{E}$, of environmental variables constitutes an assumption which makes it possible to discuss a full range of genotypic and environmental effects on P, including covariances and interactions. In the latter, neither of these is included, but P is expressed as a sum of well-defined, and therefore interpretable, uncorrelated random variables. This model is free of assumptions and therefore independent of the functional one. However, the value of accepting both, and therefore admitting the

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assumption inherent in the first, comes out in the analysis of covariances among the G_a, G_{na} and E between related individuals. It will be permissible then to replace expectation operators of the form $\xi_{P|\underline{G}}$ (see Equations [1], [4], [5] and [8]) by ones of the form $\xi_{\underline{E}|\underline{G}}$ and thereby to investigate in detail environmental effects on covariances.

III. Covariances Among Genetically and Environmentally Paired Individuals

It has been demonstrated that, for a single randomly sampled individual,

$$\xi(G_a G_{na}) = \xi(G_a E) = \xi(G_{na} E) = 0 ,$$

with the result that $v_p = v_{G_a} + v_{G_{na}} + v_E$. For randomly sampled pairs of individuals from populations of size N, very similar results hold, viz.

$$\xi(G_a G'_a) = -\frac{v_{G_a}}{N-1} , \quad \xi(G_{na} G'_{na}) = -\frac{v_{G_{na}}}{N-1} , \quad \xi(EE') = -\frac{v_E}{N-1} ,$$

$$\xi(G_a G'_{na}) = \xi(G_a E') = \xi(G_{na} E') = 0 .$$

The error in approximation in taking all to be zero is negligible for standard size human populations for which genetic effects are usually defined.

A problem occurs when pairs are not randomly sampled from the general population, but only from some subpopulation defined by pedigree or by environmental relationship among individuals. In this case, none of the above zero covariance results holds automatically. It will be possible to derive the exact form for $\xi(G_a G'_a)$ in the cases of interest for this study, but the same will seldom be possible for the remaining covariances. To illustrate this point, we consider the following very important examples.

First, for monozygotic twins with phenotypes P_1 and P_2, we know $\underline{G}_1 = \underline{G}_2$ and hence $G_1 = G_2$, $G_{a_1} = G_{a_2}$ and $G_{na_1} = G_{na_2}$. Thus,

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$$\xi(G_a G'_a) = v_{G_a}, \quad \xi(G_{na} G'_{na}) = v_{G_{na}}, \quad \xi(G_a G'_{na}) = 0 . \qquad [10]$$

If it is assumed that the twins' being reared in the same environment implies $\underline{E}_1 = \underline{E}_2$, then $E_1 = E_2$ and

$$\xi(EE') = v_E, \quad \xi(G_a E') = \xi(G_{na} E') = 0 . \qquad [11]$$

However, the assumption $\underline{E}_1 = \underline{E}_2$ often would be unrealistic because, with monozygotic twins, it implies $P(\underline{G}_1, \underline{E}_1) = P(\underline{G}_2, \underline{E}_2) \Leftrightarrow P_1 = P_2$--i.e., it implies such twins are phenotypically identical. This is certainly not the case for complex behavioral traits! Thus, the assumption $\underline{E}_1 = \underline{E}_2$ must be discarded in preference of $\underline{E}_1, \underline{E}_2$ sampled, possibly highly correlated due to common rearing of the twins, but with negligible probability of being equal.

The effect of not assuming $\underline{E}_1 = \underline{E}_2$ shows up in one of the covariances involving the environmental scores. These are

$$\xi(G_a E') = \xi_{\underline{G}}[G_a \xi_{\underline{E}|\underline{G}}(E)] = 0 \qquad [12]$$

$$\xi(G_{na} E') = \xi_{\underline{G}}[G_{na} \xi_{\underline{E}|\underline{G}}(E) = 0 \qquad [13]$$

$$\xi(EE') = \xi_{\underline{G}} \xi_{\underline{E}_1, \underline{E}_2|\underline{G}_1}(E_1 E_2) . \qquad [14]$$

The last of these can be deduced in only two cases. Firstly, if $\underline{E}_1 = \underline{E}_2$, which has been rejected, then $\underline{E}_1, \underline{E}_2|\underline{G} = \underline{E}|\underline{G}$ and $\xi(EE') = v_E$. Secondly, if $\underline{E}_1$ and $\underline{E}_2$ are conditionally independent, $\underline{E}_1, \underline{E}_2|\underline{G} = (\underline{E}_1|\underline{G})(\underline{E}_2|G)$ and, by equation [4], $\xi(EE') = 0$. This however is not realistic, even for twins reared apart, if there are any prenatal or postnatal environmental effects which occur prior to the time of separation. In general, $\xi(EE')$ will be unknown, although restricted in magnitude to the range $[0, v_E)$.

A more general example will now be given to show that the problem of nondetermined covariances is not restricted to monozygotic twin pairs.

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$\xi(G_a G'_a)$ can be deduced exactly in pairs more distantly related, but now $\xi(G_a G'_{na})$ and $\xi(G_{na} G'_{na})$ are unknown, as well as are the covariances involving environmental scores when the individuals are reared together or under similar (or dissimilar) conditions.

There are simplistic models of gene action for which $\xi(G_a G'_{na}) = 0$. They do not imply $\xi(G_{na} G'_{na}) = 0$. We will henceforth employ these results as assumptions in the ensuing development.

The covariances involving environmental scores are given by equations [12] - [14] after $\underline{E}|\underline{G}$ is replaced by $\underline{E}_2|\underline{G}_1,\underline{G}_2$ and $\underline{E}_1,\underline{E}_2|\underline{G}$ is replaced by $\underline{E}_1,\underline{E}_2|\underline{G}_1,\underline{G}_2$. The conditional distributions in the first replacement can be equated when environmental variates are independent of genotypes (i.e., $\underline{E}_2|\underline{G}_1,\underline{G}_2 = \underline{E} = \underline{E}|\underline{G}$) or when additional information on the genotype of a relative does not alter the distribution of environmental variates for individuals of a given genotype (i.e., $\underline{E}_2|\underline{G}_1,\underline{G}_2 = \underline{E}|\underline{G}$). In both cases, $\xi(G_a E') = \xi(G_{na} E') = 0$. Since the second description appears very reasonable for the case of traits influenced by many loci, we will henceforth adopt the assumption of zero covariances. There is no advantage to equating distributions in the second replacement, so we will continue to regard $\xi(EE')$ as unknown.

In summary, it has been demonstrated that equating $\xi(G_a G'_{na})$ to zero constitutes an assumption for all pairs except monozygotic twins and randomly sampled individuals. In no case other than random pairing can $\xi(G_{na} G'_{na})$ be equated to zero. The magnitude of this covariance is a maximum for monozygotic twin pairing and should decline roughly with diminishing degree of relationship. Covariances $\xi(G_a E')$ and $\xi(G_{na} E')$ are zero for monozygotic twin pairings and in special but realistic genotype x environment relationships for quantitative traits under

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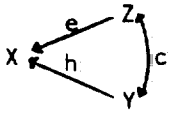
control of multiple loci. Realistically, the correlation of E with E' is never one and is zero only in randomly sampled pairs.

IV. Path Analysis for Quantitative Inheritance

All quantitative theories in which it is possible to separate genetic and environmental effects are based on analyses of covariances and hence must be mathematically equivalent to path analyses. We choose to use the latter exclusively from here on because of the facility it affords for representing relationships through pedigree.

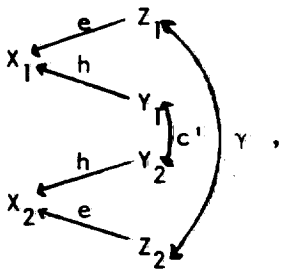
The variables X, Y and Z in a path representation of a linear model for a quantitative trait represent, respectively, standardized: phenotype, total or additive genetic score, and environmental score plus nonadditive phenotypic residual. Thus, in



, [15]

h^2 is a heritability coefficient in the broad sense if Y is standardized G, and in the narrow sense if Y is standardized G_a. In the first case, Z is standardized E; in the second, it is standardized G_{na} + E. In either case, c = 0 as a consequence of equations [4] and [8].

For purposes of analyzing phenotypes, the two path models cannot be distinguished until phenotypic correlations between individuals are introduced and the correlation paths between Y elements are specified. For example, in



, [16]

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the c' correlation path can be determined if Y is standardized G_a because it has been given that $\xi(G_a G'_a)$ can be deduced. On the other hand, it cannot be determined and must be left unspecified if Y is standardized G because it has been shown that $\xi(G_{na} G'_{na})$ cannot be deduced. Thus, there is considerable advantage to directing any correlation analysis toward an estimation of heritability in the narrow sense and a less than full separation of environmental and nonadditive genetic effects.

Notice that, independently of the choice of path models, the correlations between Z's and Y's are zero. Those common to a given X are zero by equations [5] and [8], and those associated with different X's by the assumption $\xi(G_a G'_{na}) = 0$ and the less restrictive one $\xi(G_a E') = 0$. The nonzero correlation, γ, is

$$\frac{\xi(G_{na} G'_{na}) + \xi(EE')}{v_{G_{na}} + v_E} = (1 - \alpha)\text{corr}(G_{na}, G'_{na}) + \alpha\text{corr}(E, E') , \qquad [17]$$

where $0 \leq \alpha = v_E/(v_{G_{na}} + v_E) \leq 1$.

In the path scheme where Y is the standardized additive score, c' can be expressed as a function of the degree of relationship between individuals in a sampled pair and correlations among relatives in additive scores due to assortment. Thus, for monozygotic twins, $c' = 1$; for other sibling pairs and parent-offspring, $c' = (1 + \rho)/2$; for half-siblings, $c' = (1 + 2\rho + \rho')/4$; for grandparent-offspring, $c' = (1 + \rho + 2\rho'')/4$; for uncle-nephew, $c' = (1 + \rho + 2\rho'')/4$; for first cousins, $c' = (1 + \rho + 4\rho'' + 2\rho''')/8$; etc. The appearance of primed coefficients in these solutions denotes the secondary assortative correlations among nonconsanguineous relatives of mated pairs. For example, in the half-sibling diagram

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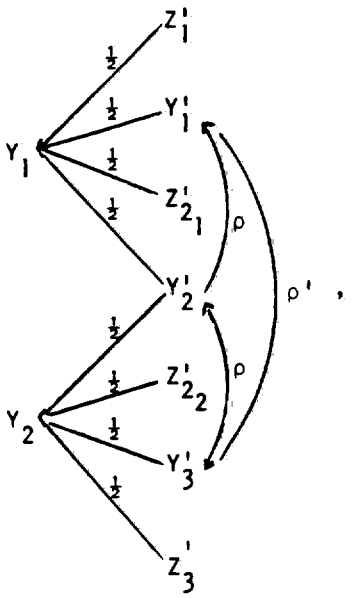

[18]

Y' variables are parental additive scores, Z' variables are segregation residuals, ρ is the correlation in additive scores due to assortment in mating, and ρ' is the correlation in additive scores resulting from phenotypic pairing of successive spouses 1 and 3 of common parent 2.

A reduction must be made in the number of primed correlations based on acceptable assumptions concerning secondary pairing. The simplest realistic assumption is that the source of secondary correlation is the relationship both individuals bear to the common member of the parental pair, i.e., the correlation is zero after linear adjustment of additive scores for the common parent. Thus, in the relationship illustrated, $\rho' = \rho^2$. Applying similar arguments, we obtain $\rho'' = \rho(1+\rho)/2$, $\rho''' = \rho^2(1+\rho)/2$ for the other cited secondary correlations.

These coefficients are entered in Table 1, given in the following section. The first set of entries is for nonconsanguineous pairs related through marriage. They give the solutions for primed correlations obtained by applying the zero partial correlation assumption. The 13 entries provide a basis for checking this assumption. The second set

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is for consanguineous pairings which are also based, as shown above, on this assumption.

V. Correlation Analysis for Children Reared in Natural Homes

Twenty-eight pairings in familial relationships spanning at most three generations and two families are listed in Table 1. The partition of a phenotypic correlation into additive genetic and residual sources is obtained in a row by multiplying h^2 and $(1 - h^2)$ by the listed coefficients and summing the resultants. For example, half-siblings with a common mother (row 21) have the following phenotypic correlation:

$$\frac{(1 + \rho)^2}{4} h^2 + (\alpha_2 + \beta_3 + \gamma_1)(1 - h^2) .$$

Coefficients of $(1 - h^2)$ have been modeled using 10 parameters to measure environmental and nonadditive genetic effects. These form the scheme:

$\alpha =$ {
- 0: unrelated prenatal environment
- α_1: related prenatal environment through sibling mothers
- α_2: related prenatal environment through common mother (different pregnancies) or monozygous twin mothers
- α_3: common prenatal environment of same pregnancy

}

$\beta =$ {
- 0: unrelated postnatal environment of individuals reared separately in natural homes and no more closely related by pedigree than first cousins
- β_1: related postnatal environment of individuals reared separately in natural homes and related by pedigree as are double first cousins.
- β_2: related postnatal environment of individuals reared

}

20100505 18

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Table 1

Relationship	Coefficient of h^2	Coefficient of $(1 - h^2)$
Nonconsanguineous pairs		
1. Mates	ρ	θ
2. Siblings-in-law through monozygous twins	ρ	$\theta(\alpha_3 + \beta_3 + \gamma_3)$
3. Siblings-in-law through dizygous twins	$\rho(1 + \rho)/2$	$\theta(\alpha_2 + \beta_3 + \gamma_2)$
4. Siblings-in-law through nontwin siblings	$\rho(1 + \rho)/2$	$\theta(\alpha_3 + \beta_3 + \gamma_2)$
5. Mother - offspring-in-law	$\rho(1 + \rho)/2$	$\theta(\alpha_1 + \beta_2 + \gamma_2)$
6. Father - offspring-in-law	$\rho(1 + \rho)/2$	$\theta(\beta_2 + \gamma_2)$
7. Mother's monozygous twin's spouse	$\rho(1 + \rho)/2$	$\theta(\alpha_1 + \beta_2 + \gamma_2)$
8. Father's monozygous twin's spouse	$\rho(1 + \rho)/2$	$\theta(\beta_2 + \gamma_2)$
9. Parent's dizygous twin's spouse	$\rho(1 + \rho)^2/4$	$\theta(\beta_1 + \gamma_1)$
10. Parent's nontwin sibling's spouse	$\rho(1 + \rho)^2/4$	$\theta(\beta_1 + \gamma_1)$
11. Successive spouses	ρ^2	θ^2
12. Siblings-in-law through monozygous twins, once removed	ρ^2	$\theta^2(\alpha_3 + \beta_3 + \gamma_3)$
13. Siblings-in-law, once removed	$\rho^2(1 + \rho)/2$	$\theta^2(\alpha_2 + \beta_3 + \gamma_2)$
Consanguineous pairs; offspring with:		
14. Monozygous twin	1	$\alpha_3 + \beta_3 + \gamma_3$
15. Dizygous twin	$(1 + \rho)/2$	$\alpha_3 + \beta_3 + \gamma_2$
16. Nontwin sibling	$(1 + \rho)/2$	$\alpha_2 + \beta_3 + \gamma_2$
17. Mother	$(1 + \rho)/2$	$\alpha_1 + \beta_2 + \gamma_2$
18. Father	$(1 + \rho)/2$	$\beta_2 + \gamma_2$

20100505 19

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Table 1 (continued)

Relationship	Coefficient of h^2	Coefficient of $(1 - h^2)$
19. Mother's monozygous twin	$(1 + \rho)/2$	$\alpha_1 + \beta_2 + \gamma_2$
20. Father's monozygous twin	$(1 + \rho)/2$	$\beta_2 + \gamma_2$
21. Half-siblings, common mother	$(1 + \rho)^2/4$	$\alpha_2 + \beta_3 + \gamma_1$
22. Half-siblings, common father	$(1 + \rho)^2/4$	$\beta_3 + \gamma_1$
23. First cousins through monozygous twin mothers	$(1 + \rho)^2/4$	$\alpha_2 + \beta_1 + \gamma_1$
24. First cousins through monozygous twin fathers	$(1 + \rho)^2/4$	$\beta_1 + \gamma_1$
25. Grandparent	$(1 + \rho)^2/4$	$\beta_1 + \gamma_1$
26. Parent's dizygous twin	$(1 + \rho)^2/4$	$\beta_1 + \gamma_1$
27. Parent's nontwin sibling	$(1 + \rho)^2/4$	$\beta_1 + \gamma_1$
28. First cousins	$(1 + \rho)^3/8$	0

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separately in natural homes and related by pedigree as are nontwin siblings

β_3: common postnatal environment of individuals reared in the same home

γ = {

0: unrelated nonadditive genetic scores of individuals no more closely related by pedigree than first cousins

γ_1: related nonadditive genetic scores of individuals related by pedigree as are double first cousins

γ_2: related nonadditive genetic scores of individuals related by pedigree as are nontwin siblings

γ_3: common nonadditive genetic scores of monozygous twins

θ = {related nonadditive genetic plus environmental scores of mates resulting from phenotypic assortment

With these, 19 different levels of correlation between residuals are obtained for the 28 pairings.

There are 12 coefficients in the correlation model, 19 distinct correlation equations, and 28 modeled relationships in all. These provide ample freedom to test the fit of the model and, if necessary, to introduce up to 7 additional coefficients, as well as to estimate all coefficients.

Without relationships involving twins, either monozygous or dizygous, one requires 10 parameters for the model, while there are 13 distinct equations and 15 modeled relationships in all. These also will provide for a test of the fit of the model and, if necessary, allow for the inclusion of up to 3 additional parameters, as well as

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forming the bases for estimating all coefficients. However, some of the most informative relationships concerning h^2 and the only information on common prenatal environment (α_3) and common nonadditive scores (γ_3) will be lost if twin relationships are removed.

Each equation in Table 1 was obtained in the following manner. A path diagram of the relationship in additive genetic scores, such as shown for half-siblings in [18], was analyzed for the correlation in these scores. For each case, secondary correlations, such as ρ' in [18], were chosen so that the partial correlation between scores unrelated by pedigree (e.g., Y_1' and Y_3') but linearly adjusted for common intermediaries (e.g., Y_2') would be zero. The resulting solution, always a function of ρ, is the coefficient entered in the h^2 column. The correlation between residuals was either worked out from first principles and minimal assumptions (for pedigree-related individuals, rows 14-28) or obtained by applying the zero partial correlation rule (when relationships are through association rather than consanguineous, rows 1-13). In the case of the former, the β and γ correlation parameters are assumed to diminish with the degree of relationship through pedigree (although in no given functional way) and to vanish altogether for individuals no more closely related than first cousins. The α parameter is assumed to depend on the pedigree relationship between mothers (vanishing for those less closely related than siblings) and the number of births per pregnancy. The extent of these assumptions can be studied in the scheme presented previously in this section.

VI. Sampling

The 13 distinct equations not based on twin relationships can be estimated from a single type of sample of family pairs. This, which we

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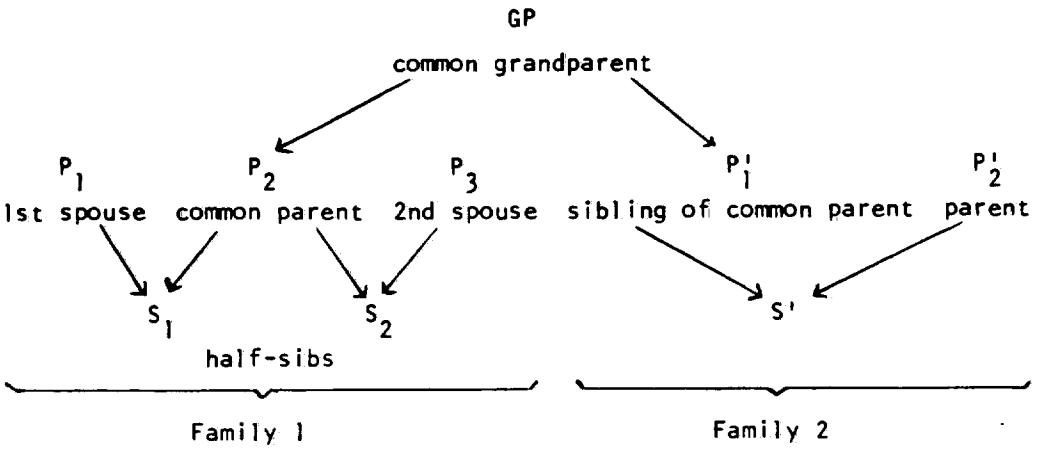
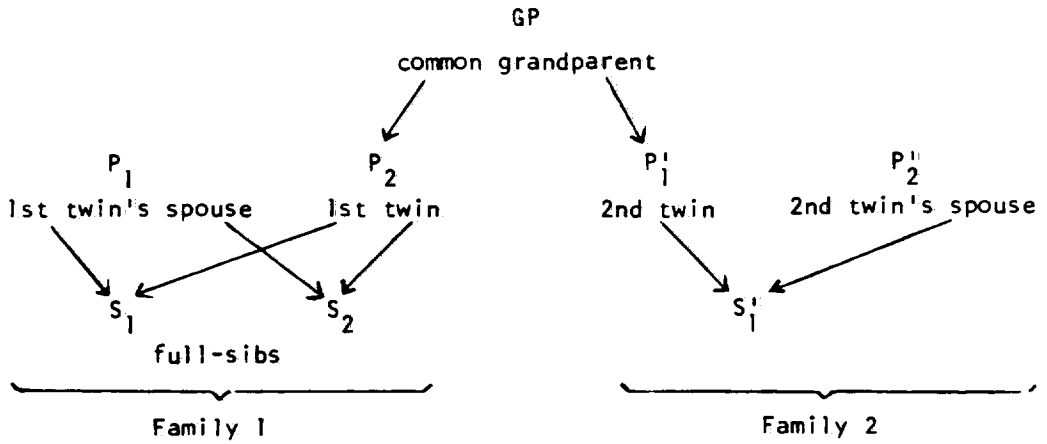
call type I, has the form:



The same family pair relationship with the multiple marriage feature replaced by twin offspring of the grandparent is called type II and provides information on the remaining distinct correlation equations. This type has the form:



It is quite important that these pairs be obtained. Without them, it is not possible to estimate α_3 and γ_3, and the separation of ρ and h^2 cannot be made nearly as effectively as it can with them.

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Preliminary calculations of the cost of conducting the field work for this study and of the precision of the data analyses are based on the following sampling schedule.

Belgium

	Type of sample	# family pairs	# interview groups	# interviewees
I:	brother parents (P_2,P_1')	25	175	225
I:	sister parents (P_2,P_1')	25	175	225
I:	male common parent (P_2) with sister (P_1')	25	175	225
I:	female common parent (P_2) with brother (P_1')	25	175	225
II:	dizygous twins	75	450	600
II:	monozygous twins	25	150	200
		200	1300	1700

The Netherlands

	Type of sample	# family pairs	# interview groups	# interviewees
I:	brother parents (P_2,P_1')	25	175	225
I:	sister parents (P_2,P_1')	25	175	225
I:	male common parent (P_2) with sister (P_1')	25	175	225
I:	female common parent (P_2) with brother (P_1')	25	175	225
		100	700	900

Sweden

	Type of sample	# family pairs	# interview groups	# interviewees
I:	mixed	25	175	225
II:	monozygous twins	75	450	600
		100	625	825

The Belgian project is the most important one in this scheme. From that geographically small, densely populated and socioeconomically diverse country, it will be possible, with the given structure of the sample, to

2010050524

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test the proposed model and to estimate all parameters with precisions which are approximately one-half those given below for the combined sample from all three countries.

The Dutch sample is planned as a safeguard. Due to the low divorce rate in predominantly Catholic Belgium, it may be difficult to obtain type I samples there. If this is the case, type III samples (in which both families involve a single marriage) will be used to fill out the 100 family pair schedule. Such samples provide useful data, but the information is not as complete as for type I. The Dutch sample would then provide the missing half-sib information, as well as a check on the extent to which the Belgian data can be extrapolated.

The Swedish sample is included for two reasons. The first is to provide twin family data to supplement the Belgian sample. There is a large Swedish twin register which makes such samples easy to draw. The second is to provide an additional check on the Belgian data to ascertain whether or not they are more than regionally characteristic.

The coefficients of variation for estimates of the various correlation equations, if sample data can be combined among countries, will range between one-halfand two percent. Those for the individual genetic and environmental parameters will be somewhat higher, in the range of three to six percent.

VII. Statistical Analyses

Summary behavioral random variables obtained from multivariate analyses, such as factor scores of a factor analysis, are usually very close to being normally distributed. Thus, samples of family pairs for a single variable of this type can be analyzed as independent, identically distributed multivariate normal vectors. For each different

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type of sample, the method of maximum likelihood can be applied to obtain efficient estimates of the correlation equations (and their identifiable components) descriptive of the relationships in that sample. The fit of the model can also be tested using likelihood methods. Estimates can then be combined linearly among samples in proportion to the quantity of information in each to obtain final efficient estimates of the genetic and environmental parameters.

The problems of testing the model and efficient estimation of the parameters are computational, not mathematical or statistical.

VIII. Adoption Studies

The value of relationships involving adopted children has not been ignored. However, the problems of obtaining information on children placed in randomly selected homes have been found exceedingly difficult to solve.

Firstly, there is the difficulty of locating such children and both their biological and foster parents. In Belgium, as in most countries, this would have to be done through the cooperation of private placement agencies. The children would have to be old enough to have had an adequate opportunity to develop a smoking behavior. Whether sufficient numbers could be found for a study based principally on adoptions is unknown but regarded as unlikely.

Secondly, there is the question of whether the foster homes are actually selected at random or if some effort is made to match child and foster parents. Our reanalysis of published data indicates that the latter appears to be the case in studies involving intelligence test scores, and this suggests that our model would have to be extended to account for such pairing.

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The third consideration is that children reared in a foster home may have a different heritability for the trait in question than those reared in a natural home. This arises from a difference in the distribution of environmental effects in the population of foster homes from that in natural homes and in genotypes of children available for adoption from those reared by their natural parents. Evidence for these differences can be found in data on intelligence scores.

Finally, if nonadditive and prenatal effects are real, adoptive relationships do not provide as clearcut estimates of genetic parameters as is frequently stated. Consider the differences shown in Table 2, in which correlations for three relationships are given under the general model and the special model of no prenatal or nonadditive effects.

Table 2

Relationship	Coefficient of h^{2} (a)	Coefficient of $(1 - h^2)$ (b)	Correlation under special model
Monozygous twins in separate foster homes	1	$\alpha_3 + \gamma_3$	h^2
Dizygous twins in separate foster homes	$(1 + \rho)/2$	$\alpha_3 + \gamma_2$	$[(1 + \rho)/2]h^2$
Nontwin siblings in separate foster homes	$(1 + \rho)/2$	$\alpha_2 + \gamma_2$	$[(1 + \rho)/2]h^2$

(a) h^2 is replaced by hh' if heritability depends on biological relationship of child to parent and one member of a pair is reared in a natural home, the other in a foster home.

(b) $(1 - h^2)$ is replaced by $[(1 - h^2)(1 - h'^2)]^{\frac{1}{2}}$ under conditions described in footnote (a).

The principal gain in using adoptive relationships is the elimination of β from the correlation equations and resulting simplification of the

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estimation problem. However, for this study, postnatal environmental effects are quite interesting, thus making adoption data of less value.

In spite of our stated reservations, it would be interesting to study samples based on adoptive relationships to determine if the difficulties we set out exist for smoking behavior. If they do not, such data would provide excellent corroborative evidence for the main study. Plans should be made to obtain small samples of this type of data if this proves feasible within Belgium, The Netherlands and/or Sweden, and if it can be fit in the existing budget.

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